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A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms

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ABSTRACT

The need to develop and improve sustainable energy resources is eminent due to the finite nature of our fossil fuels. Recent modification of laws, development of technologies and business movement has encouraged the transition to a recycling-based society. Today economy and technologies largely depend upon energy resources that are renewable as well as eco-friendly. Biogas is an environment friendly, economic and an alternative means to fossil fuel. The use of biogas is widespread in countries such as India and China. The Indian government's energy policy tries to support renewable energy by providing incentives to central and state government level. Attempts have been made to manipulate the biogas process towards industrial scale by pre and post manipulation. Pre manipulation includes maintenance of temperature, moisture, pH and microorganisms. Whereas post manipulation involves scrubbing in which water vapor, CO_2 and H_2S has been removed through activated charcoal and water shower. Bacteria and enzymes play crucial role in anaerobic digestion and they are essential for efficient process. Trials have been made by use of bio-enzymatic preparations to enhance the biogas production. This paper reviews the details about decomposition, process of anaerobic decomposition, diverse constraints behind anaerobic technology and different aspects for enhancement of biogas production through enzymes and microorganisms.

Contents

1. Introduction

Throughout the twentieth century and into the beginning of twenty first century petroleum has played an increasingly crucial role in social, economic and political history in the world. We have

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encountered major energy crisis in 1973, 1979 and 1990. All three of these crisis coincided with increased social usage of petroleum as well as political turmoil in exporting countries [16]. Therefore, it is necessary to search for an alternative source of gaseous fuel which has the potential to meet the present and future energy needs. Use of fossil fuel releases greenhouse gases into atmosphere results in global warming; this leads to seek for bioenergy. The bioenergy will have the possibility of covering more than 50% of the renewable supply of the fixed goals by the year 2020. Biofuels, in conjunction to their positive carbon balance with regards to fossil fuels, also represent a significant potential for sustainability because they can be generated from locally available renewable material. The current debate over first generation biofuels produced from food crops has pinned a lot of hope on second generation biofuels produced majorly from crop, forest residues and non-food energy crops. Policies designed to support the promotion of second generation biofuels must be carefully developed if they are to avoid unwanted consequences and potentially delay commercialization.

On the other hand, unmanaged organic fractions from farming, industry and municipalities decompose in the environment, resulting in large-scale contamination of land, water and air. Recent legislation in the United States and European countries are forcing member countries to reduce the amount of biodegradable organic waste entering landfills. Anaerobic digestion is a suitable technology to treat the solid waste and waste water and it has been considered as a waste to energy technology. The production of biogas through anaerobic digestion offers significant advantages over other forms bioenergy production. It has been evaluated as one of the most energy-efficient and environmentally beneficial technology for bioenergy production. Limitation of carbon dioxide and other emission through emission regulations, carbon taxes and subsidies on biomass energy is making anaerobic digestion a more attractive and competitive technology for waste management [40].

2. Decomposition

Decomposition is a complex and continuous process whereby the multifaceted organic structure of biological material is reduced to its mineral form. It is characterized by many biological and physical processes, including biological respiration, leaching and fragmentation [24,21]. All these process have closed relationship with each other and work synergistically. It is controlled by many factors mainly including site conditions like temperature, humidity, O_2/CO_2 concentration and substrate quality including species, size, component and position. The decomposition can be divided in to abiotic and biotic decomposition. Abiotic decomposition means degradation of a substance by chemical or physical processes. Biotic decomposition means the metabolic breakdown of materials into simpler components by living organisms typically by microorganism like bacteria, fungi, and protozoa. In decomposition there are different products are released: carbon dioxide (CO_2), energy, water, plant nutrients and resynthesized organic carbon compounds [28]. The decomposition by microorganisms takes place in either aerobic or anaerobic condition.

2.1. Aerobic decomposition

Aerobic decomposition involves the use of oxygen as an electron acceptor by microorganisms during the degradation of organic matter in to CO_2, water, nitrates and sulphates. In nature, the aerobic process is most common in areas such as the forest floor where droppings from trees and animals are converted into relatively stable organic matter. Aerobic decomposition or composting can be accomplished in pits, bins, stacks, or piles, if adequate oxygen is provided. To maintain aerobic conditions, it is necessary to add oxygen by turning the pile occasionally or by some other method. The drawback behind the aerobic decomposition is some organics cannot be decomposed efficiently. These biologically non-reactive components mainly composed of insoluble materials can account for up to 70% of the chemical oxygen demand (COD). Another issue is the fast production of biomass (sludge buildup) due to active aerobic growth powered by a sufficient oxygen supply by aeration, potentially leading to reduction in storage capacity of lagoons. These negative aspects make anaerobic decomposition as a versatile technology for the waste treatment.

2.2. Anaerobic decomposition

Anaerobic decomposition is a process by which a complex mixture of symbiotic microorganisms transforms organic materials under oxygen-free conditions into biogas, nutrients and additional cell matter, leaving salts and refractory organic matter. Raw biogas typically consists of methane (60%), carbon dioxide (40%), water vapor and trace amounts of hydrogen sulfide. Biogas is an odourless and colourless gas that burns with clear blue flame similar to that of LPG gas. Microorganisms from two biological kingdoms, the Bacteria and the Archaea carry out this process in strict anaerobic conditions [12,58]. In nature this process occurs in environments such as marshes, ponds, swamps, paddy fields, lakes, hot springs, landfills, sewage digesters, oceans and intestinal tracts of humans and animals [27]. Anaerobic digestion stabilizes the organic matter in wastewater solids, reduces pathogens and odors, and reduces the total solids by converting part of the volatile solids fraction into biogas. This process results in a product that contains stabilized solids, as well as some available forms of nutrients such as ammonia-nitrogen. The application of the anaerobic treatment process in waste management includes septic tanks, sludge digesters, industrial wastewater treatment, municipal wastewater treatment, hazardous waste management (aromatic and halogenated compounds), and agricultural waste management. Much of the fermentation used industrially to produce food and drink products, as well as home fermentation uses anaerobic digestion. Silage is produced by anaerobic digestion. Various factors like biogas potential of feedstock, design of digester, inoculum, nature of substrate, pH, temperature, loading rate, hydraulic retention time (HRT), carbon nitrogen ratio, volatile fatty acids (VFA) etc. influence the biogas production. Anaerobic digestion can be performed as a batch process or a continuous process. In batch system biomass is added to the reactor at the start of the process while in continuous digestion processes, the biomass is constantly added to the reactor. Anaerobic digestion is both a waste treatment technology, which enhances environmental quality and a sustainable energy producing technology.

2.3. Stages in anaerobic decomposition

There are four key biological and chemical stages of anaerobic digestion. The stages include hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 1).

2.3.1. Hydrolysis/liquefaction

The first stage is the depolymerisation of organic matter. During hydrolysis complex insoluble substrate such as polysaccharides are hydrolysed into smaller units by a large number of hydrolytic microorganisms (Clostridia, Micrococci, Bacteroides, Butyrivibrio, Fusobacterium, Selenomonas, Streptococcus) secreting different hydrolyzing enzymes such as cellulase, cellobiase,

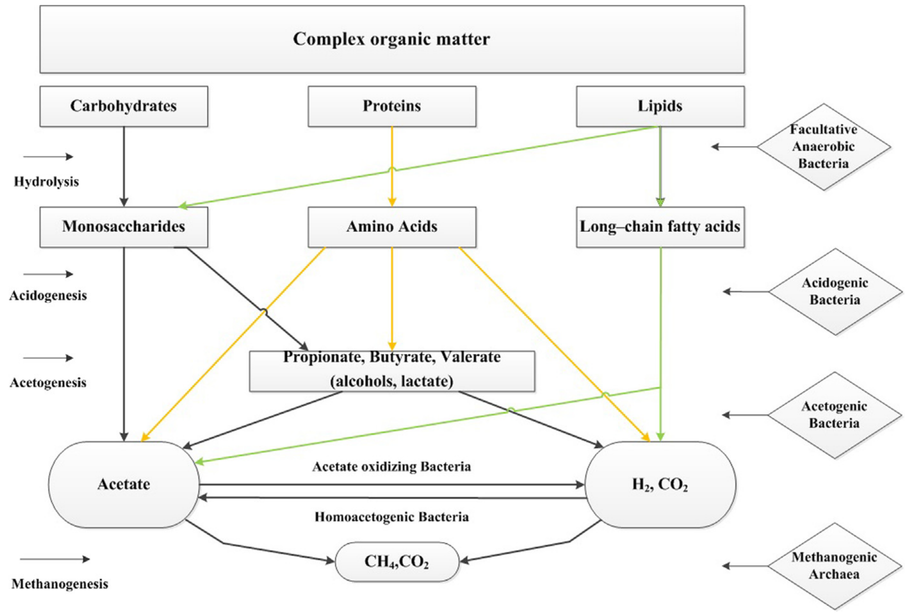


Fig. 1. Schematic representation of anaerobic decomposition.

xylanase, amylase, protease, lipase [8]. Hydrolytic reactions comprise two phases, force by extracellular enzymes secreted by bacteria which are obligate or facultative anaerobes. In the first phase a bacterial colonization takes place where the hydrolytic bacteria cover the surface of solids. Bacteria on the particle surface release enzymes and produce monomers which can be utilized by the hydrolytic bacteria themselves, as well as by the other bacteria. In the second phase the particle surface will be degraded by the bacteria at a constant depth per unit of time [57].

$$C_6H_{10}O_4+2H_2O \rightarrow C_6H_{12}O_6+2H_2 \quad (1)$$

An approximate chemical formula for the mixture of organic waste is $C_6H_{10}O_4$ [54]. Eq. (1) shows an example of hydrolysis reaction where organic waste is broken down into a simpler sugar, glucose. Hydrolysis of cellulose by the enzyme complex cellulase yield glucose, hemicellulose degradation results in monosaccharides such as xylose, glucose, galactose, arabinose and mannose [33]. The anaerobic digestion of solid lignocellulosic material and accessibility of hydrolytic microorganisms to the solid matter, constitute the rate limiting step [37]. Therefore, one strategy is pre-treatment in order to break the polymer, which prevents penetration by microorganisms or extracellular enzymes. The previous methods for pretreatment of lingocellulose involve reaction with strong acids and bases. These technologies have not proven to be commercially viable because of cost of pretreatment. The current invention utilizes a readily available and inexpensive chemical to overcome the limitations and costs of pretreatment convert lingo cellulose into a biologically degradable substrate suitable for feeding to the consortium of bacteria in an anaerobic digester. Tatsuya et al. [53] investigated the characteristics of the degradation of cellulose, soluble starch and glucose. He reported that the specific rate of substrate utilization decreased in the following order: glucose, soluble starch, acetic acids and cellulose. The rate of hydrolysis of cellulose was so low and it was shown to be a rate limiting step in overall digestion.

The United Utilities in the UK developed a specialized plug flow enzymatic hydrolysis process to pretreat the sludge before anaerobic digestion. The enzyme hydrolysis step breaks down cell wall lipoprotein structures, enhancing the digestion process. This process results in a better energy balance, enhanced digestion and increased biogas production relative to other processes [31]. Chulhwan et al. studied the efficiency of anaerobic digestion of waste activated sludge by either thermo chemical or biological hydrolysis. The thermo chemical hydrolysis showed better results than biological hydrolysis in a bench-scale operation with total chemical oxygen demand (tCOD) reduction 88.9%, volatile solid 75.5%, methane yield 0.52 m^3/kg VS and methane biogas content was 79.5% respectively [7]. The cellulose saccharification can be enhanced by cellulase using cellulose dissolution (NaOH/Urea solution, N-methylmorpholine-N-oxide, ionic liquid and 80% phosphoric acid) as a pretreatment. In comparison with conventional cellulose pretreatment processes the dissolution pretreatment exhibited a significant improvement (about 2.7–4.6 fold enhancement) on cellulose saccharification [6]. The addition of commercial enzyme preparation containing alpha amylase showed higher hydrolysis efficiency in the biological excess sludge. The mixture of two enzymes (protease: amylase = 1:3) resulted in optimum hydrolysis efficiency, the efficiency of solid hydrolysis increased from 10% (control test) to 68.43% at the temperature of 50 °C [64]. Weib et al. enhanced the biogas production by addition of hemicellulolytic bacteria immobilized on activated zeolite. The increase of methane yield is about 53% when compare to control [60].

2.3.2. Acidogenesis

Hydrolytic and acidogenic microorganisms are growing about ten times faster than methanogens. Acidogenesis is usually the fastest reaction in the anaerobic conversion of complex organic matter in liquid phase digestion [38]. During acidification of sugars, long chain fatty acids and amino acids resulting from hydrolysis are used as substrate for fermentative microorganisms (Streptococcus, Lactobacillus, Bacillus, *Escherichia coli*, Salmonella) to produce organic acids, such as acetic, propionic, butyric and other short-chain fatty acids, alcohols, H_2 and CO_2 or by anaerobic oxidizers [30,22].

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH+2CO_2 \quad (2)$$

$$C_6H_{12}O_6+2H_2 \leftrightarrow 2CH_3CH_2COOH+2H_2O \quad (3)$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (4)$$

Eqs. (2)–(4) represent three typical acidogenesis reactions [3]. In Eq. (2), glucose is converted to ethanol. Eq. (3) shows glucose is transformed to propionate and Eq. (4) shows glucose is converted to acetic acid. The transition from organic material to organic acids causes the pH of the system to drop; this condition is beneficial for acidogenic and acetogenic bacteria that prefer a slightly acidic environment, with a pH of 4.5 to 5.5. Most of the products formed in the metabolism of glucose have, as an intermediate, pyruvic acid, which is produced via the glycolytic Embden- Meyerhof-Parnas (EMP) pathway. Depending on the anaerobic microbial species present, and reactor conditions, subsequent pyruvic acid fermentation can lead to the production of a number of C_1–C_4 compounds such as volatile fatty acids (VAF_S) e.g. acetic, propionic, and butyric acids, other organic acids (formic and lactic), alcohols, Ketones and aldehydes [10]. Amino acids can also serve as energy and carbon sources for strict or facultative fermentative bacteria. Short chain VFA_S (C_2–C_5, straight chain or branched) are generated via reductive deamination of aliphatic amino acids, specific fermentative pathways of individual amino acids, or an oxidation-reduction reaction between pairs of amino acids, known as Stickland reaction [14,42]. The accumulation of electron sinks such as lactate, ethanol, propionate, butyrate and higher VFA_S are responsible for the bacteria to increase hydrogen concentration in the medium [47]. The concentration and proportion of individual VFA_S produced in the acidogenic stage is important in the overall performance of the anaerobic digestion system since, acetic and butyric acids are the preferred precursors for methane formation [26]. Hydrolysis and acidogenesis can be enhanced by increasing the operating temperature however; acetogenesis is adversely affected by high operating temperature. If the system is heated to enhance hydrolysis and acidogenesis, the resulting volatile acid production can overwhelm the ability of the slower reacting acetogenic and methanogenic bacteria to convert the volatile acids, resulting in increased pH and inhibited acetogenesis and methanogenesis [5].

Elefsiniotis et al. studied the effect of solid retention time (SRT) on the acid-phase anaerobic digestion of primary sludge. He showed that a constant hydraulic retention time (HRT) of 12 h, variation in solids retention time from 10–20 days resulted in a slight increase in VFA_S production but a shorter SRT (5 days) resulted in a drastic drop in acid production [15]. Higher contribution of substrate may cause lower methane yield due to higher consumption in the hydrolytic-acidogenic stage. The higher loading provokes a decrease in the acidification yield, probably due to the fact that the acidogenic bacteria could have been affected and inhibited at the highest organic loading rate [32].

2.3.3. Acetogenesis

Acetogenic bacteria are strict anaerobes, have optimum pH around 6 and isolated mostly from anoxic habitats and utilize a pathway (the acetyl coenzyme A pathway) that contain enzymes extremely sensitive to O_2 [62]. They are slow growing, sensitive to fluctuations in organic loadings and environmental changes. They require long lag periods for adjust to new environmental conditions [63]. Increasing hydrogen concentration in the liquid will lead to accumulation of electron sinks (lactate ethanol, propionate, butyrate and higher volatile acids) which cannot be consumed directly by the methanogens and should be degraded further by the obligate hydrogen producing acetogenic bacteria and the process is referred to as acetogenesis [4]. The obligate hydrogen-producing acetogenic bacteria (*Syntrophomonas wolfeii*, *Syntrophobacter wolinii*) degrade the electron sinks to acetate, carbon dioxide and hydrogen. This transition is important for the successful production of biogas [36]. Acetogens make syntrophic associations with hydrogen-consuming methanogens because they depend on low hydrogen partial pressure for their degradation [46,49].

$$H_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (5)$$

$$C_6\ H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (6)$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (7)$$

Eq. (5) represents the conversion of propionate to acetate, only achievable at low hydrogen pressure. In Eq. (6) Glucose is converted to acetate. The acetogens cannot convert ethanol directly to methane and carbon dioxide; first it must convert the ethanol to acetic acid and consequent release of molecular hydrogen. Eq. (7) shows the ethanol is transformed to acetate.

In the termite gut acetogens are the dominant hydrogen sinks. Leadbetter et al. [35] reported that methanogens and acetogens inhabit the termite gut can tolerate and consume traces of O_2. Arno et al. [1] grew the acetogens (*Sporomusa silvacetica*, *Moorella thermoacetica*, *Clostridum magnum*, *Acetobacterium woodii* and *Thermoanaerobacter kivui*) in both semisolid and liquid cultivation media containing O_2. Low concentrations of O_2 caused a lag phase in growth but did not alter the ability of these acetogens to synthesize via the acetyl coenzyme A pathway. The first acetogenic genome sequenced and annotated is Moorella thermoacetica. It is 2.6 mega base genome and 70% of the genes have been assigned tentative functions [45]. Acetogens enhance biodegradative capacity by coupling the oxidation of hydrogen gas to the reduction of CO_2 to acetate [44]. A novel process was developed and demonstrated that coupled syntrophic acetogenesis with homoacetogenesis reaction was able to enhance the acetate production from high strength synthetic wastewater [65].

2.3.4. Methanogenesis

Methane is produced as a metabolic byproduct in anoxic conditions by methanogenic microorganisms belongs to Archaea. Methanogen has an unusual type of metabolism, because they use H_2/CO_2, formate, methylated C1 compounds or acetate as energy and carbon sources for growth. Methane production is occurs in two ways either by means of cleavage of acetic acid molecules to generate carbon dioxide and methane or by reduction of carbon dioxide with hydrogen [39].

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (8)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (9)$$

The hydrogenotrophic methanogenesis is the most common metabolic pathway where CO_2 and H_2 are converted to methane (Eq. (8)). Besides H_2, most of the hydrogenotrophs can also use formate as the major electron donor [18]. In this case, the formate dehydrogenase oxidizes four molecules of formate to CO_2 before one molecule of CO_2 is decomposed to methane. During hydrogenotrophic methanogenesis the CO_2 is stepwise reduced to methane by special coenzymes (methanofuran, tetrahydromethanoptein, coenzyme M) through the formyl, methylene and methyl levels. The key enzyme of this process is the methyl- coenzyme M reductase which reduces methyl-coenzyme M to methane whereby the oxidized coenzyme M forms a heterodisulfide complex M heterodissulfide complex with coenzyme B [13]. In the second type of methanogenesis, the aceticlastic methanogenesis, acetate is directly converted to methane (Eq. (9)). The carboxyl-group of the acetate is oxidized to CO_2 whereby the methyl-group is reduced to methane [17]. Two major pathways of acetate degradation are known which only differ in the first step. One group of acetotrophic methanogens, the Methanosarcinaceae, uses the acetate kinase phosphotransacetylase system for activating acetate to acetyl-coenzyme A. Methanosaetaceae the second group

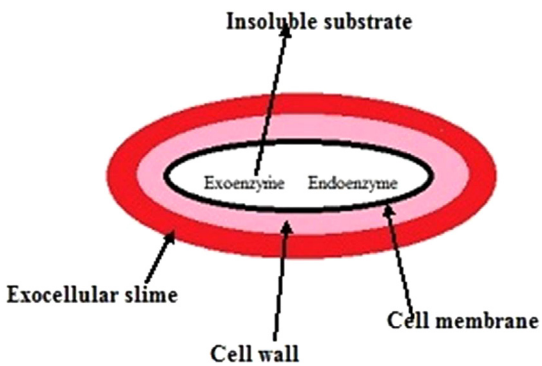


Fig. 2. Schematic representation of two types of enzymes (exo and endo enzymes) involved in substrate degradation.

of acetate converts, adenosine monophosphate forming acetyl-coenzyme A synthatase for this reaction [51]. The hydrogen consuming methanogens (*Methannospirillum hungatei*, *Methanoculles receptaculi*) are fast growing than the aceticlastic methanogens (*Methanosarcina thermophila*). The maximum doubling time for hydrogenotrophic methanogens has been estimated to be six hours compared with slowing growing aceticlastic methanogens which takes 2.6 days. Hydrogen utilizing methanogens are more resistant to environmental changes than aceticlastic methanogens. Methanogens are very sensitive to changes and prefer slightly alkaline environment [19]. If the pH fall below six methanogenic bacteria cannot survive. Methanogenesis is the rate controlling portion of the anaerobic process [9].

Kalle et al. [29] improved the methanogenesis through the use of mixed cultures (cellulolytic culture C_{35} mixed with methanogen M_{35}) isolated from biogas digester. Lalov et al. [34] improved the biogas production by covalent immobilization of a methanogenic consortium on to a granulated polymeric support (poly acrylonitrile- acrylamide). The inhibitory effect of oxygen was reduced by immobilizing the methanogenic consortium.

3. Enzymatic ability to degrade substrate

Bacteria degrade substrate through the use of enzymes. Enzymes are proteinaceous molecules that catalyze biochemical reactions. Two types of enzymes involved in substrate degradation are endoenzymes and exoenzymes. Endoenzymes are produced in the cell and degrade soluble substrate within the cell. Exoenzymes are also produced in the cell but are released through the “slime” coating the cell to the insoluble substrate attached to the slime. Once in contact with the substrate the exoenzymes solubilize particulate and colloidal substrate. Once solubilized, these substrates enter the cell and are degraded by endoenzymes (Fig. 2). The production of exoenzymes and solubilization of particulate and colloidal substrates usually take several hours. All bacteria produce endoenzymes, but not all bacteria produce exoenzymes. No bacterium produces all the exoenzymes that are needed to degrade the large variety of particulate and colloidal substrates that are found in sludges and wastewaters. Each exoenzyme as well as each endoenzyme degrades only a specific substrate or a group of substrates. Therefore a large and diverse community of bacteria needed to ensure that the proper types of exoenzymes and endoenzymes are available for degradation of the substrates present. The relative abundance of bacteria within an anaerobic digester often is greater than 10^{16} cells per milliliter. This population consists of saccharolytic bacteria (approximately 10^5 cells/ml), and methane-forming bacteria (approximately 10^8 cells/ml) [20].

To respond to the increased demand for biofuels, advanced biochemical processes using enzymes are being developed, which are gaining increased global attention. Research in this field aims to improving efficiency, and reducing negative environmental impacts, of production processes, in addition to enhancing the quality of the produced biogas. Enzymes have been employed to overcome the drawbacks associated with the use of conventional chemical catalyst. For example, the use of enzymes for the hydrolysis of cellulose to produce fermentable sugar for ethanol production, the utility cost of enzymatic hydrolysis is much lower when compared to the alternative methods of acidic hydrolysis. A number of authors have reported significant improvement in biogas production when crude and commercial enzymes are used in the pretreatment of complex organic matter. There have been studies on the improvement of biogas production from lignocellulolytic materials, one of the largest and renewable sources of energy on earth, after pretreatment with cellulases and cellulase-producing microorganisms. Improved methane yield has been reported in the literature when the lipid-rich wastewaters are pretreated with lipases and lipase-producing microorganisms. The enzymatic treatment of mixed sludge by added enzymes prior to anaerobic digestion has been shown to result in improved degradation of the sludge and an increase in methane production [41]. A Canadian company, SunOpta, markets a patent technology known as “Steam Explosion” to pretreat cellulosic biomass, overcoming its “recalcitrance” to make cellulose and hemicellulose accessible to enzymes for conversion into fermentable sugars. Strategies for enzyme dosing to enhance anaerobic digestion of the different complex organic rich materials have been investigated.

4. Microbial strains enhance the biogas production

Strains of some bacteria and fungi have been found to enhance biogas production by stimulating the activity of particular enzymes. Cellulolytic strains like Actinomycetes and mixed consortia have been found to improve biogas production in the range of 8.4–44% from the cattle dung [55,2]. Three fibrolytic bacteria isolated from sheep's colon using cellulose (b), xylan (c), and lignin (d) as selective substrates. These isolates were then used both in pure and mixed culture with cattle cellulolytic bacteria (a). The highest in vitro biogas and methane production was obtained from a-c-d co-culture addition. The a-c-d co-culture as inoculum for in vitro feces fermentation increase the overall gas production 56.36% and methane content 18.09% compared to the natural fermentation by feces microbes [59]. Dohanyos et al. examined the use of cell lysate as a stimulating agent in anaerobic degradation of municipal raw sludge, excess activated sludge and their mixture. The effect of lysate is caused by the still remaining activity of released enzymes and by the stimulating properties of other compounds that are present inside the cells [11]. Research on anaerobic degradation of cellulosic wastes by rumen microorganisms for enhanced production of methane and ethanol has shown clear correlation between the lignin content of several wastes, natural materials and their degradability by rumen microorganism [25]. Payel et al. [43] prepared eleven different microbial consortia with concomitant enzymatic activity for the effective degradation of organic kitchen waste. The degradation of organic waste by the bacterial consortia was highly significant. It reduces the time span of degradation and produces no foul odor. Sharda et al. [50] carried out the experiment by using three different microbial consortiums like consortia “A”, “B” and “C” to find out the suitable consortia for maximum biogas generation. Consortia “B” contain strict and facultative anaerobic bacteria Bacteroides, Peptostreptococcus, Clostridium and Propionibacterium. Consortia “C” contain methanogenic bacteria like *Methanobacterium formicicum*, *Methanobrevibacter ruminantium*, *Methanisarcina frisia*, *Methanothrix soehngenii*. Consortia “A” contain all the 8 isolates. The highest

methane concentration (76%) was obtained from consortia "C" containing four different methanogenic bacteria when compared to consortia "A" (23%) and consortia "B" (1%).

5. Genetic engineering of microorganism and enzymes for enhanced of biogas production

Genetic engineering plays a major in all aspects of biotechnology and also in biofuel production. Current research is focused on using the power of genetic engineering to improve the biogas production. This is done by manipulating genes in specific pathways and or incorporating specific DNA fragments into target species. In 2010, a genetically engineered yeast strain was developed to produce its own cellulose-digesting enzymes. Assuming this technology can be scaled to industrial levels; it would eliminate one or more steps of cellulolysis, reducing both the time required and costs of production. Recombinant strains of *Saccharomyces cerevisiae* have been genetically engineered to carry out simultaneous saccharification and fermentation (SSF) to produce extracellular endoglucanase and glucosidase that are able to ferment cellulose and hemicellulose to 6-carbon and 5-carbon sugars and subsequent to ethanol [48,56,23,61]. Recently, engineered yeasts have been described efficiently fermenting xylose, and arabinose and even both together. Current and future research trends are directed towards the developments and applications of genetically engineered organism to tackle the challenges encountered from conventional naturally occurring strains. In a lot of cases we are still at the point of developing tools to manipulate the target species, but recent breakthroughs are showing a lot of promise on a lab to pilot scale.

Research attention is also focused on genetic engineering in enzymes production. Recently, genes of various enzymes have successfully been cloned, and genes are promised to be cloned rapidly in the coming years. The use of recombinant DNA technology to produce large quantities of recombinant enzymes will help lower the enzymes costs. In addition, protein engineering will help to create novel enzyme proteins that are most resistant and highly thermo-stable. The introduction of cheap enzymes with enhanced activities and resilience should change the economic balance in favor of enzyme use [52]. The fungus Trichoderma reesei is used by Iogen Corporation to secrete "specially engineered enzymes" for an enzymatic hydrolysis process. Other enzyme companies, such as Dyadic International, are developing genetically engineered fungi which would produce large volumes of cellulase, xylanase and hemicellulase enzymes, which can be used to convert agricultural residues such as corn stover, distiller grains, wheat straw and sugarcane bagasse and energy crops such as switch grass into fermentable sugars.

6. Future trends

The European Commission has set the goal by 2020, that 20% of the energy consumed should come from renewable energy sources, as well as 10% the energy consumed within the transport sector. Biogas is a renewable energy source, contains 55–65% methane which cannot be used as vehicle fuel without upgrading. Attempts were being made to increase the methane content in biogas through optimize the techniques used for biogas production. Biomass pretreatment and hydrolysis are areas in need of drastic improvement for economic production of biogas from complex organic matter such as lignocellulosic material and sewage sludge. The production of biogas has been greatly improved by new technologies; there are still challenges that need further investigation. These challenges include, (1) in hydrolysis process, the complex insoluble organic materials are hydrolyzed by extracellular enzymes is a rate—limiting step of the process. Significant amount of information has been published on the kinetics of hydrolysis. In most cases, the experimental data have not been used for the development of appropriate kinetic models. Therefore there is a need to development of a standard for the hydrolysis and acidogenesis steps that will allow proper exploitation of past information and appropriate focusing on future research endeavors. (2) Lignocellulosic biomass represents a rather unused source for biogas. Many factors like lignin content, crystallinity of cellulose and particle size limits the digestibility of the hemicellulose and cellulose present in the cellulosic biomass. Enzyme pretreatment has as a goal to improve the digestibility of the lignocellulosic biomass. The high cost of commercial enzyme production, however, still limits application of enzymatic hydrolysis in full-scale biogas production plants. The extensive research is now being directed towards these aspects to improve the biogas production. It is reported that using iron oxide nanoparticles in anaerobic digester enhance the biogas production. It is envisaged that nano materials from renewable biowaste will be the main focus for future research.

7. Conclusion

According to Olduvai theory, the life expectancy of the industrial civilization was found to be ended in 2030. Korpela, 2006 has debated on the need for alternative energy sources to address concerns over the decision of fossil fuels. The search for a replacement of fossil fuels must be prioritized to ensure the future of human civilization. Bioenergy will be the most significant renewable energy source, because it offers an economical attractive and alternative to fossil fuels. Aerobic decomposition is found to be faster but their economics is not comparable with anaerobic decomposition. Anaerobic process have many advantages over the aerobic process because, low consumption of energy, low sludge production, smaller space requirements, reduction in waste volume, production of biofertiliser and valuable soil conditioners. The European Parliament Committee on Industry, External Trade, Research and Energy calls on to increase efforts within the research on new technologies of biogas as a biofuel. The success of biogas production will come from the availability at low costs and the wide variety of usable forms of biogas for the production of heat, stream, electricity and for the utilization as a vehicle fuel. However, the present knowledge on anaerobic decomposition is not sufficient to use it at industrial scale with complete control of all its parameters and factors. For an increasing dissemination of biogas plants further improvements of the process efficiency, perfect designing of anaerobic digester for efficient recycling of organic matter and the development of new technologies for process monitoring and process control are necessary. The influence of microbial community structure on process stability, important bacterial strains actively involved in the degradation, requires further efforts and must be analyzed in detail. Furthermore, a clear understanding of the organization and activities of diverse microbial community is crucial for optimization of their performance and attainment of the stable operation process.

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